Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 36 (2012) 150 – 153

Procedia Engineering

www.elsevier.com/locate/procedia

IUMRS-ICA 2011

Transmission Electron Microscopy Observation of Antibody

Marina Kamogawa[a], Junichi Shimanuki[b],

Takachika Azuma[c], Akikazu Murakami[c], and Takashi Ishiguro[a,*]

[a]*Tokyo University of Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278-8510, Japan*

[b]*Research Department, NISSAN ARC, LTD., 1, Natsushima-cho, Yokosuka, Kanagawa 237-0061, Japan*

[c]*Research Institute for Biological Sciences (RIBS), Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278-8510, Japan*

Abstract

High resolution electron microscopy (TEM) observation of an antibody, which is stained with sodium phosphotungstate solution, has been performed at accelerating voltage of 200 kV. In order to reduce background noise due to superposed substrate image, we observed antibodies standing on the edge of the mica substrate in the vacuum.

Two typical images have been taken successfully. One shows Y-shaped contrast. The other image, which is thought to be a side view image, shows constricted part corresponding to hinge region. Both lengths of Fab (fragment, antigen binding) region and Fc (fagment, crystallizable) region are measured directly by referring lattice fringes of mica.

Keywords: Transmission electron microscopy; ionic liquid; monoclonal antibody; stain; sodium phosphotungstate; mica

* Corresponding author. Tel.: +81-4-7122-9668; Fax: +81-4-7122-9668.

E-mail address: ishiguro@rs.noda.tus.ac.jp.

1877-7058 © 2012 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.

doi:10.1016/j.proeng.2012.03.023

1. Introduction

Nanoscale observation of biological soft matter such as protein and DNA, etc. has been studied intensively in recent years. As it is well known, the expression of function of biological soft matter usually depends on its three-dimensional conformation. Therefore, the direct nanoscale observation of individual matter is important technique which should be developed. In this study, we choose an antibody as observation object. Antibody connects specifically with antigen but it has not yet to be revealed what fine structure has influence on antigen-antibody reaction. To clarify the mechanism, nanoscale observation of the antibody structure is important.

From the viewpoint of the resolution, TEM is one of the excellent choices. However, it has been difficult to observe biological matter, e.g., biological macromolecules such as antibody by TEM. Because they are usually composed of light elements such as H, C, N, and O and Coulomb's potential for the electron beam is small, the contrast of TEM image becomes weak fundamentally. To avoid this difficulty, negative staining method is widely-used [1]. Then, Y-shaped images of antibody have already been reported [2]. However, resolution of the images has not been enough because of both low accelerating voltage, e.g., from 80 to 120 kV, and low magnification [1]. Also, negative staining makes quite strong background images and often causes vaguely-outlined image of the object.

People usually use amorphous carbon (a-C) film for the specimen support substrate. However, the potential of a-C film is comparable in magnitude to or larger than that of biological object. Therefore, removing salt-and-pepper noise due to a-C film from the image is also necessary. An ideal situation is "nothing in particular". In other words, observation of the biological object being supported in the vacuum is the most suitable situation. Therefore we observed antibodies standing on the edge of the mica substrate in the vacuum. Thus, we succeeded in taking a high resolution image of individual antibody without background.

2. Experimental

2.1 Preparation of IgG monoclonal antibodies

The monoclonal antibody (mAb) used in the present study was anti-Hen's Egg Lysozyme mAb (IgG1). The purified mAb were dialyzed against phosphate-buffered saline (PBS). The Fab and Fc fragments of IgG1 mAb were prepared by digestion with papain, and the $F(ab')_2$ fragments were prepared by digestion with pepsin. The protein concentration was determined at an optical density (OD) of wavelength 280 nm and was calculated using molar absorption coefficients of 2.1×10^8 ($cm^2 \cdot mol^{-1}$) for an intact mAb, 8.0×10^7 ($cm^2 \cdot mol^{-1}$) for Fab, 1.6×10^8 ($cm^2 \cdot mol^{-1}$) for $F(ab')_2$ and 6.0×10^7 ($cm^2 \cdot mol^{-1}$) for Fc [3].

2.2 Electron microscopy

IgG molecules, stored in PBS (pH 7.4) with 0.05 wt% of sodium azide, and were diluted to a concentration of 0.489 mg/ml. It was dropped on freshly cleaved mica sheet and was kept for 30 min. The mica sheet was rinsed out with ultra-pure water for 3 min, and then stained with 0.04 (g/ml) sodium phosphotungstate solution for 10 min, and rinsed out with ultra-pure water for 3 min. Finally, the mica sheet was sandwiched by a 75/100-double mesh copper grid (Okenshoji Co., Ltd.) applied 1-Butyl-3-methylimidazolium tetrafluoroborate (Kanto Chemical Co., Inc.) as a conduction treatment. The specimens were examined with a Tecnai G2F20 electron microscope (FEI Co.), operated at an accelerating voltage of 200 kV and fitted with a GIF2000 Imaging Filter (Gatan Inc.). Exposure time was 10 to 15 s.

3. Results and discussion

Figure 1 shows TEM image of IgG molecules. Upper half region corresponds to vacuum. Lower half region corresponds to mica substrate, which may be coated by a layer of laying antibodies. Although resolution is not enough because of somewhat deep under-focus, two Y-shaped contrasts with height about 10 nm from the edge can be seen in the vacuum region. This shape is typical form of the antibody. Hinging angle between Fab regions is about 35 degrees.

Figure 2 also shows another image of antibody which might be corresponding to the side view of antibody in Fig. 1. The resolution is better than that of Fig. 1. At lower part, there is lattice fringes with a spacing of 0.45 nm corresponding to 110 reflection of crystal mica (monoclinic, a_0=0.51988 nm, b_0= 0.90266 nm, c_0=2.01058 nm, beta=95.782deg) [4]. Referring to this scale, we can measure the length of antibody.

In Fig. 2, constricted part indicated by arrow with “h” is thought to be a hinge region. The part between “h” and “Fc-b” corresponds to Fc region. The upper part between “h” and two “Fab-t” is thought to be the Fab regions partially overlapped each other in the image. At the top of Fab indicated by “Fab-t”, there is a bended small part with a size about 1.6 nm. Hinging angle between Fab regions is not evaluated from Fig. 2. However it can be deduced that the length of Fab region is at least 5.3 nm. If we assume hinging angle estimated by Fig. 1, the length of Fab region is estimated to be about 2.5 nm. In addition the length of Fc region is at least 3.1 nm. Width of Fc region is not constant. Width of narrow constriction is about 1.2 nm. On the other hand, width of wide part is about 2.5 nm.

For the first time, our present high-resolution TEM observation has successfully revealed the shape and the numerical size of single antibody molecule in the nanometer scale.

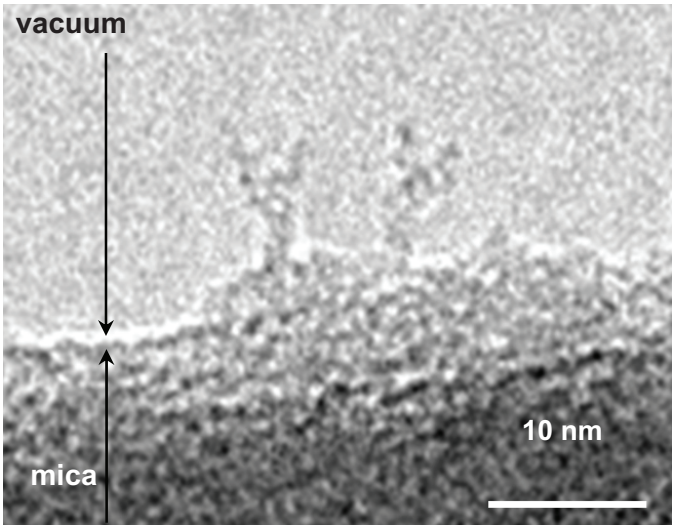

Fig. 1. TEM image of IgG antibody. Characteristic Y-shaped images can be seen.

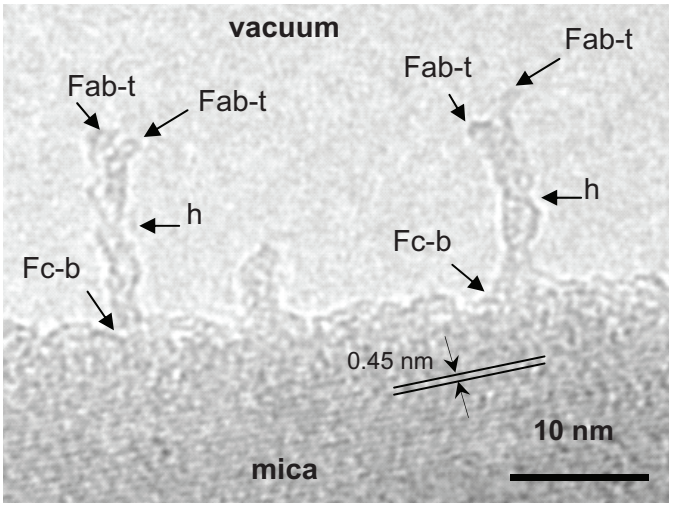

Fig. 2. High-resolution TEM image of IgG antibody (side view).

Acknowledgements

This work was supported by both a project of Center for Technologies against Cancer (2009-2013) and a project of Research Center for RNA Science (2010-2004) in Tokyo University of Science. These centers are funded by Program for Development of Strategic Research Center in Private Universities supported by the Ministry of Education, Culture, Sports, Science & Technology in Japan (MEXT).

References

[1] Kiselev N. A., Sherman M. B., and Tsuprun V. L., *Electron Microsc. Rer.*, 1990; 3: 43-72.

[2] Oda M., Uchiyama S., Noda M., Nishi Y., Koga M., Mayanagi K, Robinson C. V, Fukui K., Kobayashi Y., Morikawa K, and Azuma T., *Mol. Immunol.*, 2009; 47: 357-364.

[3] Oda M., Kozono H., Morii H, and Azuma T., *International Immunology*, 2003; 15: 417-426.

[4] Richardson, S. M., Richardson, J. W., *American Mineralogist*, 1982; 67: 69-75.